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Tyler M. Dohlman

*Iowa State University*, [tdohlman@iastate.edu](mailto:tdohlman@iastate.edu)

Marianna Jahnke

*Iowa State University*, [marianna@iastate.edu](mailto:marianna@iastate.edu)

James West

*Iowa State University*

Patrick E. Phillips

*Iowa State University*, [ovadoc@iastate.edu](mailto:ovadoc@iastate.edu)

Patrick J. Gunn

*Iowa State University*, [pgunn@iastate.edu](mailto:pgunn@iastate.edu)

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# Effects of Label-Dose Permethrin Administration on Reproductive Function and Embryo Quality on Superovulated Beef Heifers

## A.S. Leaflet R3050

Tyler Dohlman, Assistant Professor,  
Marianna Jahnke, Lecturer/Embryologist,  
James West, Senior Clinician,  
Patrick Phillips, Senior Clinician,  
Veterinary and Diagnostic Production Animal Medicine,  
Iowa State University;  
Patrick Gunn, Assistant Professor,  
Department of Animal Science, Iowa State University

### Summary and Implications

Commercial pyrethroid pour-ons are commonly applied in cow-calf operations to eliminate the potential for insect borne diseases and to improve productivity. However, recent literature has focused on potential negative reproductive effects in the bull after exposure to pyrethroids. While the female bovine has been primarily neglected from the debated pyrethroid concern on reproduction, literature in mice and rats have reported potential endocrine disruption of sex steroids resulting from pyrethroid exposure, with potential detrimental effects on female fertility. The objective was to study the effects of a commercial pyrethroid-based pour-on product, permethrin, on reproductive performance in superovulated beef heifers by assessing steroid biosynthesis and embryo quality. It was hypothesized that exposure to pyrethroid pour-on at label dose would cause minimal effects on embryo quantity/quality and steroidogenesis in the female bovine. Results from this study revealed pyrethroid-treated heifers had a tendency for reduced progesterone, but embryo quantity and quality were not affected compared to controls.

### Introduction

Pyrethroid exposure has been implicated to disrupt important reproductive and endocrine functions. In addition, endocrine disruption is speculated to influence the hypothalamic-pituitary-gonadal axis and impair the necessary feedback mechanisms of hormones that provide the required stability to regulate normal reproductive physiology. Previous observational findings have claimed that bulls exposed to pyrethroids have decreased reproductive function. However, recent literature has refuted that claim.

It is important to note that endocrine disruption is not believed to be sex specific, and thus likely to potentially affect female reproductive physiology by inhibiting normal reproductive functions. Previous research has indicated pyrethroids may inhibit progesterone concentrations by

down-regulating expression of cP450scc and StAR. In addition, literature using mice and rats as the model have shown delay in puberty in females exposed to pyrethroid substances. However, there are postulated thoughts that endocrine disruption chemicals (EDC) could also stimulate changes in the reproductive tract that impede sperm migration, sperm adhesion, capacitation, zona binding, acrosomal reaction, or penetration into the oocyte or the competency for maturation of a developing embryos. Pre-implantation losses with reduction of implantation sites has been reported with rats receiving orally administered pyrethroids in early gestation, which could imply that exposure to pyrethroids could develop a hostile environment or cause abnormal synchronization of embryo development or implantation.

The overall reproductive effects of pyrethroid exposure to female cattle have not been studied in as much detail as the bull. The objective of this study was to elucidate the effects of a commercial pyrethroid-based pour-on product, permethrin, on reproductive performance in superovulated beef heifers by assessing steroid synthesis and embryo quality. It was hypothesized that exposure to pyrethroid pour-on at label dose would cause minimal effects on reproductive parameters in the female bovine.

### Materials and Methods

Non-pregnant, purebred Simmental and crossbred yearling beef heifers ( $n=10$ ;  $418 \pm 33$  kg;  $5.5 \pm 0.2$  BCS) were used in this study. Prior to treatment, all heifers were subjected to a trans-rectal reproductive ultrasound examination to confirm normal ovarian activity and cyclicity. At that time, initial body weight (BW) and body conditioning scores (BCS) were recorded and heifers were blocked by breed and BW. Heifers were assigned to either 1) a saline control (CON;  $n=5$ ) or 2) a permethrin pour-on treatment group (PYR;  $n=5$ ). The PYR heifers received a one-time label dose of permethrin (5% permethrin and 5% piperonyl butoxide, 3 mL per 45kg BW up to a maximum of 30 mL) for lice and fly control. The CON group received the same volume of saline. Both products were administered on the topline of the heifers. Treatment groups were housed one pen per treatment to avoid cross-contamination. All heifers received the same environmental and nutritional treatment before and after treatment.

Treatments were initiated at the start of superovulation protocol. All heifers were subjected to superstimulation by utilizing a timed, 17-d, CIDR-based protocol with GnRH and PGF2 $\alpha$  with decreasing dosages of FSH administered twice daily for 4 days (Experimental Design, Figure 1).

Heifers were artificially inseminated (AI) twice either at onset of estrus or by the timed-AI and additionally 12 hrs later by same technician with one unit of frozen semen, at each insemination, from a single bull collection. A dose of GnRH was given at time of second breeding. At 6.5 d post-timed-AI, trans-rectal ultrasound was performed to assess corpus luteum (CL) number, number of unovulated follicles, and total ovarian structures (CL and unovulated follicles). Immediately following ultrasound, non-surgical embryo recovery was performed. All recovered embryos were evaluated according to International Embryo Transfer Society (IETA) standards by blinded American Embryo Transfer Association (AETA) certified personnel.

To determine potential long-term effects of permethrin on embryo quality and steroid biosynthesis, an identical superstimulation protocol was initiated again 34 d post-treatment with embryo recovery performed 51 d post-treatment. On the second flush, one heifer had abnormal oviduct pathology and embryo data was not used.

Blood was collected via coccygeal tail vein at insertion of CIDR™, standing estrus, and day of embryo recovery to evaluate baseline (basal) estradiol-17β, peak estradiol-17β, and progesterone (P4) concentrations, respectively. Blood was centrifuged and the plasma was utilized for the hormone analysis using radioimmunoassay (RIA).

**Figure 1. Experimental Design**

Day	Time	Administrations	Ultrasound/ Blood Collection
-33	am	.....	U/S, BW + BCS
0	am	CIDR™-insertion + 625 mcg PGF2α + Ultraboss® or Saline Treatment	Basal Estradiol
4	am	150 mcg GnRH	.....
5	pm	40 mg FSH	.....
6	am/pm	40 mg F SH, 30 mg FSH	.....
7	am/pm	30 mg FSH, 30 mg FSH	.....
8	am	30 mg FSH	.....
8	pm	20 mg FSH + 625 mcg PGF2α	.....
9	am	20 mg FSH + 625 mcg PGF2α + CIDR™-removal	.....
10	pm	AI	Peak Estradiol
11	am	150 mcg GnRH + AI	Peak Estradiol
17	am	Embryo Recovery + 625 mcg PGF2α (post-flush)	Progesterone + U/S
34	am	Repeat d0 Protocol (Except pour-on treatments)	Basal Estradiol
51	am	Embryo Recovery + PGF2α (post-flush)	Progesterone + U/S

Data was analyzed using SAS 9.3 (SAS Institute Inc., Cary, NC) for a 2 x 2 factorial arrangement. Binary data were analyzed using the GLIMMIX procedure of SAS, while remaining, continuous reproductive and embryo parameters were analyzed using the MIXED procedure of SAS. The final model included the main effects of treatment and period (flush) and the appropriate interaction. Heifer per flush was the experimental unit and statistical significance was acknowledged at  $P \leq 0.05$ , and  $0.05 < P \leq 0.10$  was considered a tendency approaching significance.

## Results and Discussion

### Embryo quality

Total embryos recovered did not differ due to treatment ( $P = 0.30$ ), but did decrease in flush 2 compared to flush 1 ( $P = 0.02$ ). In addition, there was a treatment x flush interaction for total embryos recovered ( $P = 0.02$ ; Table 1, as CON heifers had more embryos recovered in first flush and reduced embryos in the second flush compared to PYR heifers (Table 1). Embryo quality grades and flush success did not differ due to treatment ( $P \geq 0.16$ ). However, CON heifers had an increase in unfertilized oocytes compared to PYR heifers ( $P \leq 0.05$ ). Irrespective of treatment, quality grade 1 embryos and transferable quality embryos (TQE) decreased in flush two compared to flush one ( $P \leq 0.05$ ). In addition, total unfertilized oocytes was greater in CON heifers than PYR heifers ( $P = 0.04$ ). Due to greater unfertilized oocytes in CON heifers there was a subsequent treatment x flush interaction for quality Grade 4 embryos ( $P \leq 0.02$ ).

### Hormone analysis

Progesterone (P4) concentrations on the days of embryo collection were not different between treatments; however, PYR heifers had a weak decreasing trend in total P4 concentration ( $P = 0.15$ ) and a tendency for reduced P4 per corpus luteum ( $P = 0.06$ ; Table 2). Estradiol concentration per ovulated follicle (CL) and per total ovarian structure (CL and unovulated follicles), as determined by ultrasound at embryo recovery, was greater in flush two than flush one ( $P \leq 0.02$ ,  $P \leq 0.03$ ; respectively) but did not differ due to treatment ( $P \geq 0.23$ ).

In conclusion, these data indicate that a single use of topical 5% permethrin at label dose in superovulated heifers resulted in a weak tendency for reduced progesterone concentrations, but not to a degree that impacts embryo production or quality. Permethrin exposure did not elicit any reproductive toxicity on folliculogenesis or maturation.

**Table 1. Embryo flushing characteristics for super-stimulated yearling beef heifers after treatment with a pyrethroid pour-on**

Item	Treatment <sup>1</sup>				SEM <sup>2</sup>	P-Value <sup>2</sup>		
	CON		PYR			TRT	Flush	TxF
Total Embryos	17.0	7.75	9.8	9.8	1.88	0.30	0.02	0.02
Embryo Quality Grade <sup>3</sup> (%) <sup>4</sup>								
1	4.0 (29.5)	2.25 (33.8)	5.4 (59.3)	3.0 (38.0)	0.83 (0.12)	0.32 (0.24)	0.04 (0.15)	0.83 (0.18)
2	1.4 (12.5)	0.5 (5.8)	2.2 (17.3)	1.2 (15.4)	0.59 (0.05)	0.38 (0.33)	0.27 (0.56)	0.95 (0.74)
TQE <sup>5</sup>	5.4 (42.0)	2.75 (39.7)	7.6 (76.5)	4.2 (53.5)	1.32 (0.14)	0.30 (0.19)	0.05 (0.08)	0.91 (0.45)
3	1.6 (7.3)	0.75 (11.3)	0.4 (4.0)	0.8 (6.3)	0.37 (0.02)	0.29 (0.22)	0.67 (0.34)	0.26 (0.80)
4	10.0 (50.8)	4.25 (49.0)	1.6 (17.5)	4.8 (40.2)	2.12 (0.12)	0.16 (0.20)	0.67 (0.14)	0.02 (0.29)
Degenerate <sup>6</sup>	3.2 (16.7)	1.0 (11.7)	1.2 (13.5)	4.2 (34.2)	1.09 (0.70)	0.72 (0.35)	0.76 (0.42)	0.10 (0.21)
UFO <sup>7</sup>	6.8 (34.1)	3.0 (33.8)	0.4 (4.0)	0.6 (6.0)	1.40 (0.08)	0.04 (0.02)	0.14 (0.71)	0.10 (0.95)
Flush Sucess <sup>8</sup>	0.80	0.25	0.80	0.60	1.08	0.50	0.13	0.50

<sup>1</sup> CON = Saline Control; PYR= UltraBoss® at labeled recommendation, F1 = First embryo flushing (17 days after initial treatment), F2 = Second embryo flushing (51 days after initial treatment).

<sup>2</sup> P-values of main effects of treatment and flush and the treatment x flush interaction (P ≤ 0.05; considered statistically significant).

<sup>3</sup> Average numbers of embryos graded by International Embryo Transfer Society (IETS) guidelines: 1 = Excellent or good, 2 = Fair, 3 = poor, 4 = dead or degenerate.

<sup>4</sup> Percentage of total embryos by quality grades.

<sup>5</sup> Transferable quality embryos: embryos that are of satisfactory quality to freeze and transfer. TQE = Grade 1 and Grade 2.

<sup>6</sup> Non-viable or dead embryos.

<sup>7</sup> Unfertilized oocytes.

<sup>8</sup> Percentage of animals that had ≥ 5 TQE (average of industry standards).

**Table 2. Hormonal, ultrasound, and embryo flushing characteristics for super-stimulated yearling beef heifers after treatment with a pyrethroid pour-on**

Item	Treatment <sup>1</sup>				SEM <sup>2</sup>	P-Value <sup>2</sup>		
	CON		PYR			TRT	Flush	TxF
	F1	F2	F1	F2				
Total Structures <sup>3</sup>	19.4	15.2	15.2	15.2	3.95	0.59	0.17	0.17
CL Total <sup>4</sup>	18.0	12.8	13.8	12.8	2.47	0.56	0.12	0.27
Unovulated Total <sup>5</sup>	1.4	2.4	1.4	2.4	0.58	1.00	0.21	1.00
Progesterone (ng/ml) <sup>6</sup>	94.03	82.94	42.19	32.00	22.63	0.15	0.31	0.96
P4/CL Ratio	4.94	4.98	2.86	2.48	0.74	0.06	0.76	0.70
Resting Estradiol (pg/ml) <sup>7</sup>	2.71	2.02	1.71	2.23	0.21	0.21	0.79	0.07
Peak Estradiol (pg/ml) <sup>8</sup>	42.83	50.58	25.52	47.57	6.93	0.33	0.15	0.47
Estradiol/CL Ratio	2.41	5.07	1.88	3.66	0.57	0.26	0.02	0.60
Estradiol/Total Structure Ratio	2.22	3.94	1.70	2.96	0.41	0.23	0.03	0.71
Recovery Rate <sup>9</sup>	96.0	89.0	74.0	77.0	0.12	0.35	0.87	0.69

<sup>1</sup> CON = Saline Control; PYR= UltraBoss® at labeled recommendation, F1 = First embryo flushing (17 days after initial treatment), F2 = Second embryo flushing (51 days after initial treatment).

<sup>2</sup> P-values of main effects of treatment and flush and the treatment x flush interaction ( $P \leq 0.05$ ; considered statistically significant).

<sup>3</sup> Counted corpus lutea (CL) and unovulated structures from both ovaries via rectal ultrasound at embryo recovery.

<sup>4</sup> Counted corpus lutea (CL) from both ovaries via rectal ultrasound at embryo recovery.

<sup>5</sup> Counted unovulated structures from both ovaries via rectal ultrasound at embryo recovery.

<sup>6</sup> Taken at embryo recovery by venipuncture of caudal tail vein.

<sup>7</sup> Taken prior to initiation of CIDR protocol by venipuncture of caudal tail vein.

<sup>8</sup> Taken during standing estrus or at timed artificial insemination (TAI) by venipuncture of caudal tail vein.

<sup>9</sup> Number of total embryos divided by total number of corpus lutea (CL).